# ASHRAE Research Project Report
# RP-1072

## Detection and Removal of Gaseous Effluents and By products of Fungal Growth that Affect Indoor Environments

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ASHRAE

Shaping Tomorrow's
Built Environment Today

**FINAL REPORT**

**Detection and Removal of Gaseous Effluents**

**and**

**Byproducts of Fungal Growth that Affect Indoor Environments**

**ASHRAE 1072-TRP**

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**of Fungal Growth that Affect Indoor Environments**

## 1.0    EXECUTIVE SUMMARY AND CONCLUSIONS

## 1.1    SUMMARY

*ASHRAE RFP OBJECTIVES 1 & 2: Review and evaluate the information on microbial volatile organic compounds and the potential for their control based on gas phase air filtration equipment.*

This report contains a review of the scientific issues surrounding the issue of microbial volatile organic compounds (MVOCs) together with a perspective on microbial contamination of buildings. Fungi are the common microbial contaminants of buildings and building systems (e.g. HVAC). A relatively short list of filamentous fungi (often called molds) occurs in water-damaged buildings in North America. Although there are now many studies on volatiles production by such fungi, the important species occurring in American buildings have not been reliably studied. Many studies in the literature on MVOCs have serious methodological problems.

The fungi that occur in buildings produce roughly the same mass of volatile compounds per unit weight of fungal growth. The volatiles produced comprise a mixture of compounds that are common to many species and are also industrial chemicals. In addition, such fungi produce compounds that are genera or species specific. These differences have not been adequately studied.

Methods of analysis of MVOCs reported by different groups are similar in that Gas Chromatography/Mass Spectrometry (CG/MS) is used, usually with thermal desorption. There have been few efforts to determine the collection efficiencies, recoveries and

analytical errors involved in field studies, including the impact of relative humidity on sample collection. Assumptions have been made that MVOCs will be collected and determined with similar accuracy and precision to volatile man-made chemicals of similar character. Only one moderate-sized field study has been reported where the MVOC measurements were quantitative and strictly comparable between buildings.

Because fungi mainly emit volatiles, which are also industrial chemicals, field practice (mainly in Europe) is to visually compare patterns of a short list of common MVOCs to conjecture whether a building has significant fungal contamination. This is an insensitive tool compared to other methods for evaluating mold contamination and hence has not been widely applied in the US.

Technology being developed to manage the international space station air quality includes the development of small mass spectrometers suitable for the detection of volatile chemicals. More study of the volatiles from molds isolated from buildings in the US, new statistical approaches to the analysis of volatiles patterns coupled with studies of analytical performance of collection methods can be justified on the basis of our review.  These would result in detectors in air handling systems that would provide early detection of fungal contamination of large buildings.

Current public policy, i.e. advice from governments and cognizant authorities such as AIHA and ACGIH, requires that significant mold growth on building materials or building systems be removed. It is known that exposure to the spores of fungi growing on building materials can result in disease. It is thought possible, but not known, that microbial volatile organic compounds might make a contribution to the symptoms observed in buildings with significant mold problems.  MVOCs virtually never comprise the sole exposure. There is no basis to propose studies to determine the efficiency of gas phase filters to remove MVOCs from building air.

ASHRAE RFP OBJECTIVES 3 & 4: "Determine which organisms deserve further study and what methodological issues must be resolved."

Table 1(pg 15), Part I of this report provides an extensive list of fungi that are common in American buildings and residences together with information on which species have had reliable studies of volatiles production. A shorter list, emphasizing the species that have been repeatedly isolated from contaminated building materials includes *Chaetomium globosum, Penicillium viridicatum, Eurotium herbariorum, P. aurantiogriseum, P. citrinum, Stachybotrys chartarum, Aspergillus sydowii, P. commune, Eurotium repens, A. versicolor, Paecilomyces variottii and Cladosporium sphaerospermum*. Large amounts of volatiles can be collected from these species in all-glass fermentation systems to produce reliable (i.e. not limited by the amount of fungal biomass produced) species patterns. Statistical methods for determining species-specific patterns within mixtures can be applied to determine the sensitivity of this approach for early detection of fungal growth in buildings with HVAC systems. Studies on the accuracy and precision of field sampling methods are also needed to fulfil this objective.

There are two allied objectives that could be pursued to support the goals above. These include developing a database on MVOCs that are normally found in the outdoor air at grade level around buildings, and sampling for MVOCs in buildings with known amounts of mold growth.

*ASHRAE RFP OBJECTIVE 5: "Identify health impacts of MVOCs."*

Consistent with the recent ACGIH Bioaerosols Committee publication (Macher et al. 1999), we find only suggestive evidence that MVOCs contribute in a material way to the health effects in moldy buildings. Resolution of their possible contribution is not currently possible because of inadequate methods of exposure characterization.

## 1.2    CONCLUSIONS

Active microbial growth results in the production and release of secondary metabolites.  Many of these compounds are objectionable and have very low odor thresholds.

There is insufficient evidence at present to judge the potential health effects of exposure to MVOCs.

The available evidence indicates that molds have unique MVOC profiles.  These are potentially very useful indicators of the type of mold growth in a building. Additional work is needed however to characterize more of the molds that commonly occurs in buildings.

Recent advances in analytical technology suggest that significant gains are possible in detection, analysis and pattern recognition of MVOC samples taken from buildings.   This will require application of pattern recognition software.

The release of MVOCs from microbial growth in a building can often be detected, either analytically or as odors by the occupants.   This MVOC release may serve as an early indication of moisture problems in a building.   Analytical detection with improved methodology may allow monitoring of building environments and timely correction of moisture issues.

**ASHRAE Research Project 1072-TRP**

**Detection and Removal of Gaseous Effluents and Byproducts**

**of Fungal Growth that Affect Indoor Environments**

## 2.0    BACKGROUND

## 2.1    JUSTIFICATION

Active microbial growth (molds, mildew, bacteria) produces volatile organic compounds (VOCs).  These microbial VOCs (MVOCs) cause the musty, mildewy or earthy odors that are associated with damp basements or garden soil.  MVOCs are produced regardless if the growth is on decaying material or on building materials than have been impacted by water or moisture.  MVOCs include a variety of chemical classes including alcohols, ketones, organic acids, and heterocyclic compounds, among others.  Many of these have extremely low odor thresholds, and many can be quite objectionable.

Specific MVOCs are often produced by multiple species of molds, which complicates the interpretation of MVOC data if compounds are considered singly.  Different species of molds have been shown to produce specific mixtures, or profiles, of MVOCs, which should prove useful to reveal which mold(s) are growing in a building.  There are currently very few MVOC profiles available though, from many of the molds that commonly occur in buildings.  Characterizing these profiles would facilitate the interpretation of MVOC sample results from complaint buildings.

Much of what is known of MVOCs is from studies of food spoilage molds.  Fortunately, much of this information is applicable to building mold problems, at least indirectly.  There are a number of other molds that are important in buildings that have not been studied as sources of MVOCs. There is a significant need for MVOC information from numerous molds that are important in indoor environments.

In occupied buildings with microbial growth, there often are complaints about air quality and frequently about health effects.  The odor of MVOCs is certainly a signal that alerts occupants to the presence of microbial growth and often is the original source of complaints.  These complaints often precipitate building investigations.  Furthermore, MVOC exposures may be related to the health complaints, although this has not yet been established.

Thus, it is useful to building owners and operators to understand where and how these pollutants (MVOCs) are released, which molds are likely to be sources and what are the best analytical tools for MVOCs.  This information may in turn help develop strategies to prevent MVOC release, or to use MVOC measures as an indicator of building performance.

## 2.2    OBJECTIVES

ASHRAE 1072-TRP was proposed as a project with two phases.  This report covers the first phase.  The objectives of Phase I of ASHRAE 1072-TRP included two tasks.  These were to prepare a review of the available literature on MVOCs, especially with relation to buildings, and to prepare a detailed work plan for Phase II.  This report contains the literature review and the work plan for Phase II.

## 3.0    ASHRAE 1072-TRP TASK 1: REVIEW OF LITERATURE CONTAINING MICROBIAL VOLATILE ORGANIC COMPOUNDS (MVOCs)

The following manuscript reviews and discusses the published literature on MVOCs. The emphasis was on MVOCs from molds that are known to contaminate buildings, although selected other papers are discussed where pertinent. All significant papers that we were aware of, or could find reference to, were included in the review. All other papers on MVOCs that could be located through the available libraries were also included. This is a comprehensive review on MVOCs, especially regarding MVOCs in buildings.

This review is organized into two general sections. The first covers literature on the biology and production of MVOCs. The second section of the review concerns field studies that have used MVOC sampling in buildings with known or suspected mold problems.

**MICROBIAL VOLATILE ORGANIC COMPOUNDS WITH EMPHASIS ON THOSE ARISING FROM FILAMENTOUS FUNGAL CONTAMINANTS OF BUILDINGS**

### 3.1    PART I:   BIOLOGY OF METABOLITE PRODUCTION BY FUNGI

#### 3.1.1   THE NATURE OF FUNGAL SECONDARY METABOLITES

Some fungi produce soluble and volatile metabolites that are toxic to competitors. Other fungi and bacteria will not grow in the presence of such compounds. Herbivorous animals will avoid or are killed by some fungal metabolites thus preserving the food substrate for the dominant fungus. This is called interference competition  (Wicklow 1981). Volatile organic compounds (VOCs) inhibit the growth of plant pathogenic (Hamilton-Kemp et al. 1992) and wood decay fungi (Bruce et al. 1984; 1996). Induction

of sexual reproduction in *Phytophthora* has been reported by *Trichoderma* volatiles. (Brasier et al. 1993). VOCs from fungi are also known to attract insects that are essential in transporting fungi to new food sources (Lin and Phelen 1992).

The genes for secondary compounds are typically located together on the chromosome, suggesting that horizontal gene transfers have occurred. There are many examples where similar pathways exist for chemical components that cross not just species that are closely related but even between bacteria and fungi (Jarvis & Miller 1996).

All soluble and most volatile metabolites that mediate interference competition are "secondary" metabolites, that is, compounds that are produced after one or more nutrients become limiting (Bu'Lock 1975; 1980; Miller et al. 1994 for secondary metabolites, Ito et al. 1990 for MVOCs).  It is very important to separate conditions that favor the growth of the fungi from issues of secondary metabolism gleaned from in vitro studies.  Primary metabolites are compounds produced after materials exterior to the cell are absorbed or transported inside the cell, broken to low molecular weight compounds and metabolized to compounds such as acetate, amino acids, glutamic acid, etc. (Figure 1).  This is called primary metabolism and the process uses widely occurring enzyme pathways.  Secondary metabolites are produced from one or more primary metabolites and are produced via secondary metabolism.  This uses enzyme pathways that are unique to a single species or group of related species.  The occurrence of metabolites from fungi is entirely governed by the existence of conditions that favor the growth of the fungi concerned.

Fungal mycelia are thread-like structures with a 3-4 μm diameter that comprise the fungal colony. With exceptions, only the terminal few cells of a mycelium are biologically active.  It is unambiguously known that secondary compounds are produced by the

*Figure 1. Generalized scheme of the pathways of fungal volatiles production. Primary metabolism breaks down cellulose (and other complex substances) in building materials as these substances are converted through the citric acid cycle into primary metabolites. These primary metabolites then feed into pathways of secondary metabolism, which are unique to specific groups of microbes. Some of these pathways produce volatile compounds such as isobutanol, 1-octen-3-ol, geosmin ($C_{12}H_{22}O$) (4a(2H)-Naphthalonol,octahydro-4,8a-dimethyl-,(4.alpha.,4a.alpha.,8a.beta.) and others.*
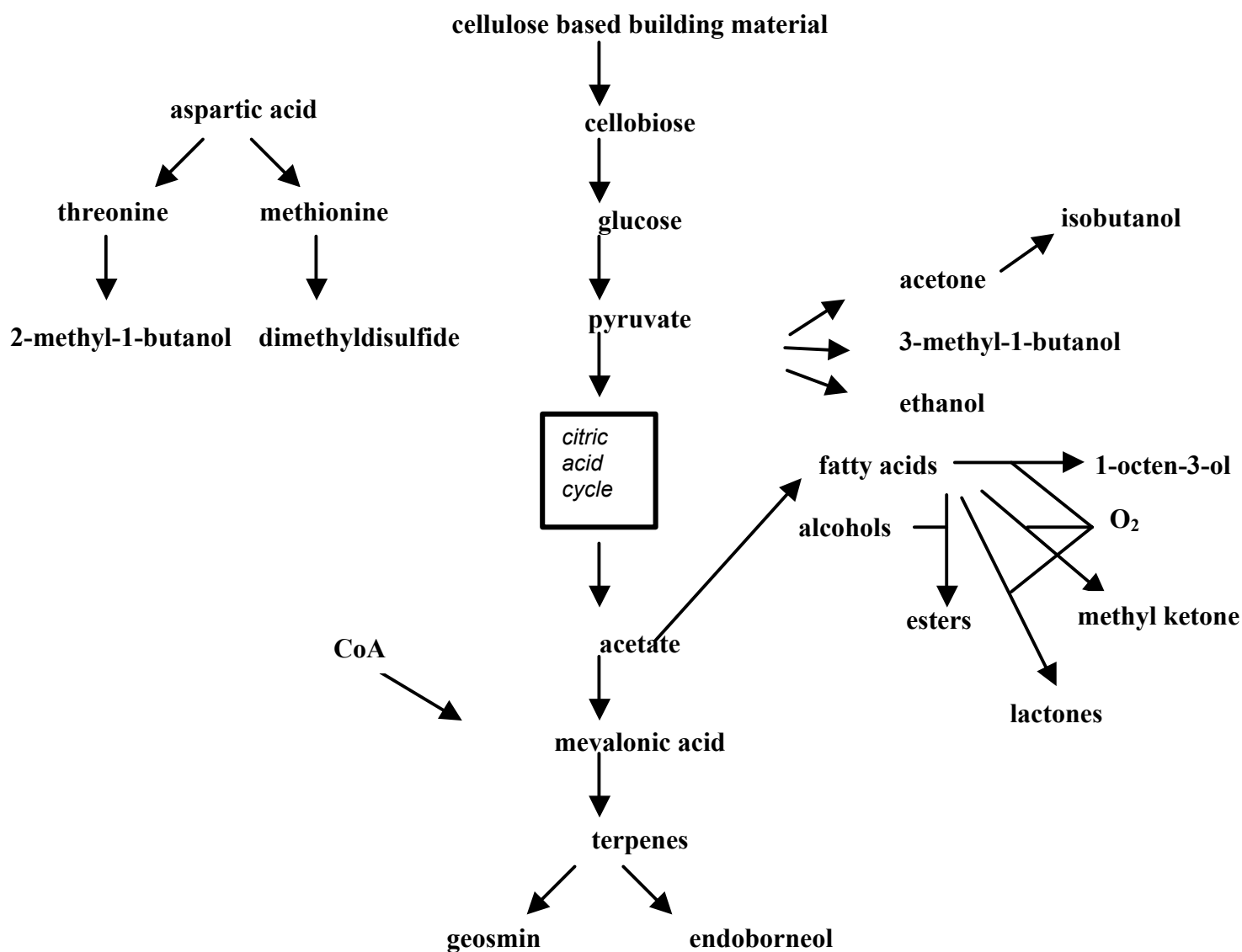
penultimate cells of each mycelium (Miller and Greenhalgh, 1988).  This means that the production of a secondary metabolite occurs on the micro level of each individual thread-like structure. The accumulation (release) of secondary compounds including many fungal VOCs occurs as the result of millions of cells on the substrate concerned. Detection of secondary compounds is a function of total fungal biomass rather than of time. That is, metabolites are produced within hours of germination and the total amount is a function of the total amount of cells.  Detection methods for metabolites are incapable of determining very small amounts of compound.  From this problem arose the common misunderstanding that fungal colonies produce secondary compounds only upon reaching some critical biomass or in response to some peculiar environmental circumstances (see Miller & Greenhalgh 1986).

Under conditions seen in a given environment, different fungal species are favored as diseases of crop plants or as saprophytes on stored crops (Miller 1995) or building materials (Flannigan & Miller 1999). When the conditions favor the growth of fungi, it is an invariable rule that one or more of the compounds for which the fungus has the genetic potential are produced.  Table 1 illustrates some fungi that are common on building materials. These fungi are therefore adapted to growth on, for example, wallboard by virtue of their tolerance to different water contents, salts content and substrate (food) preference.

A signal feature of fungi is that wild strains of some taxa have the genetic potential to produce several families of compounds, some of which overlap across species, and some of which are species specific. The pattern of compounds produced by wild strains is sometimes called a chemotype  (Larsen et al. 1995; Miller et al 1991). With modern studies of such fungi, it has been appreciated that they can produce many compounds, often from different biosynthetic families. The ecological significance of the occurrence of these mixtures has been a fertile area of study in recent years. As it relates to human

and animal toxicology or toxicity to microbes, the potency of the contaminated material is due to the mixtures present (Miller 1995b; Prelusky et al. 1994). Although there are many studies that purport to show large variances in the ability of wild strains to produce secondary metabolites within their chemotype, these always have fatal methodological errors. It is common for there to be a loss of genetic potential for pathways for both primary and secondary metabolites during the process of isolation of strains. It is an invariable rule that, in nature, if fungi grow, the colonies will contain one or more of the families of compounds for which they have the genetic potential.

**Table 1.** Common Species Found in Buildings

| Common Species Found in Buildings | | | |
|---|---|---|---|
| MVOCs Studied | | No MVOC Studies Reported | |
| Species | MVOC Reference | Species | Comment |
| *Aspergillus versicolor* | 3, 9, 11 | *Absidia corymbifera* | a |
| *Paecilomyces variottii* | 1, 9 | *Acremonium strictum* | c |
| *Penicillium aurantiogriseum* | 2, 7, 8 | *Acrodontium* sp. | b |
| *Penicillium brevicompactum* | 1, 3, 7 | *Alternaria alternata* | a |
| *Penicillium chrysogenum* | 6, 7 | *Arthrinium* sp. | b |
| *Penicillium citrinum* | 6, 7 | *Aspergillus penicilloides* | d |
| *Penicillium commune* | 9 | *Aspergillus sydowii* | a |
| *Penicillium decumbens* | 5, 7 | *Aureobasidium pullulans* | a, c, d |
| *Penicillium fellutanum* | 1 | *Botrytis cinerea* | a |
| *Penicillium glabrum* | 3 | *Chaetomium globosum* | a |
| *Penicillium olsonii* | 7 | *Chrysonilia* sp. | b |
| *Penicillium purpurogenum* | 7 | *Cladosporium cladosporioides* | a, d |
| *Penicillium viridicatum* | 6 | *Cladosporium herbarum* | a, d |
| *Trichoderma viride* | 1, 10 | *Cladosporium macrocarpum* | d |
| | | *Cladosporium sphaerospermum* | a, d |
| | | *Curvularia* sp. | b |
| | | *Doratomyces* sp. | b |
| | | *Emericella nidulans* | a |
| | | *Epicoccum nigrum* | a |
| | | *Eurotium amstelodami* | a, d |
| | | *Eurotium herbariorum* | a, d |
| | | *Eurotium rubrum* | a |
| | | *Exophiala jeanselmei* | a |

| MVOCs Studied | | No MVOC Studies Reported | |
| --- | --- | --- | --- |
| Species | MVOC Reference | Species | Comment |
| | | *Fusarium culmorum* | a |
| | | *Geomyces pannorum* | a, c |
| | | *Gliocladium* sp. | b |
| | | *Gonatobotrys* sp. | b |
| | | *Graphium* sp. | b |
| | | *Harzia* sp. | b |
| | | *Humicola* sp. | b |
| | | *Microsporum* sp. | b |
| | | *Mucor plumbeus* | a |
| | | *Nigrospora* sp. | b |
| | | *Oedocephalum* sp. | b |
| | | *Oidiodendron tenuisssimum* | a |
| | | *Ovularia* sp. | b |
| | | *Penicillium jensenii* | d |
| | | *Pestalotia* sp. | b |
| | | *Phialophora* sp. | a |
| | | *Phoma herbarum* | c |
| | | *Pithomyces* sp. | b |
| | | *Rhizopus stolonifer* | a |
| | | *Scopulariopsis brevicaulis* | a |
| | | *Scopulariopsis fusca* | a |
| | | *Serpula* sp. | b |
| | | *Sistotrema brinkmannii* | c |
| | | *Stachybotrys chartarum* | a |
| | | *Stemphylium* sp. | b |
| | | *Trichothecium* sp. | b |
| | | *Trichurus* sp. | b |
| | | *Tritirachium* sp. | b |
| | | *Ulocladium botrytis* | a |
| | | *Ulocladium chartarum* | a |
| | | *Verticillium* sp. | b |

Fungal list after Flannigan & Miller 1999; 1. Anon. 1986; 2. Borjesson et al. 1979; 3. Borjesson et al. 1992; 5. Halim et al. 1975; 6. Kaminski et al. 1974; 7. Larsen & Frisvad

1995; 8. Saito et al. 1979; 9. Sunneson et al. 1995; 10. Wheatley et al. 1997; 11. Willkins et al. 1999. Some additional studies are reported in Ammann 1999.

a: common in buildings (Samson et al. 1994)
b: common but occurring less frequently in buildings (Samson et al. 1994)
c: >33% of dwellings (Hunter et al. 1988)
d: mean indoor/outdoor ratio significantly elevated (Verhoeff et al. 1992)

In nature, the distribution of fungal species on crops or building materials depends on water content of the material. Environmental conditions hence determine which toxins will be present (Flannigan & Miller 1999; Miller 1995). In culture, or in the field, which metabolite families will then dominate in the substrate depends on $O_2$, pH, osmotic tension ($a_w$) and sometimes temperature.

An example of this is *Stachybotrys chartarum*. This fungus produces at least four families of compounds: a*tranones, macrocyclic trichothecenes, spirolactones* and *cyclosporin-like* compounds (Jarvis et al. 1995; Hinkley et al. 1999; Sakamoto et al, 1993). There appear to be two *chemotypes* present from North American strains: those that produce all of the families and those that do not produce trichothecenes but the others (Jarvis, personal communication).

## 3.1.2 VOLATILES PRODUCTION BY MOLDS

A number of critical experiments have indicated, as predicted by the biochemistry involved, that the absolute amount of volatiles produced is a function of numbers of fungal cells. Experiments done on *Penicillium fellutanum, P. brevicompactum, Trichoderma viride* and *Paecilomyces variotti* (all strains isolated from building materials) indicated that weight of volatiles production on a glucose, salts, peptone/yeast extract/malt extract medium between these taxa was similar on a mycelial dry weight basis (Anon, 1986). These experiments were done in all-glass

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fermentation systems in still cultures where the headspace gasses could be collected and the dry weights of the cells determined afterwards.  In addition, the experimental set-up provided large headspace concentrations such that the absolute amounts of volatiles could be determined.  This may be important because there is some evidence that scavenging volatiles from headspaces improves volatiles yield and hence detectability (Kuhne & Sprecher 1987). In nature, the headspace would of course be infinite. Volatiles from these four taxa were collected on Tenax GC and determined after thermal desorption on a high-resolution mass spectrometer. The above strains were grown on wet, irradiated foam insulation and volatiles were collected and analyzed as above. Volatiles produced on insulation and in liquid culture media were similar (Anon. 1986; see also Ammann 1999).  Volatiles data from *Paecilomyces variotti* are reported in Table 2.

A second, critical, experiment that offers information on the amount of volatiles produced per unit biomass was reported by Borjesson et al. (1989). They found that volatiles production evolved on a natural substrate (autoclaved wheat grains) from *Eurotium amstelodomi, Aspergillus flavus, Fusarium culmorum and Penicillium aurantiogriseum* were in similar amounts. This was based on the sum of measured amounts of individual compounds (in some case, classes) when adjusted for growth based on $CO_2$ production (another absolute measure of growth). *E. amstelodomi* and *P. aurantiogriseum* are building material-associated fungi. The same author produced an excellent study where volatiles were compared to total $CO_2$ production and to ergosterol, an unambiguous measure of fungal biomass (Gessner & Newell 1997). If one species is removed *(P. brevicompactum)* from their study, the production of volatiles was highly correlated to biomass and $CO_2$ production (Borjesson et al. 1992).

A less accurate, but useful, experiment giving information on the relationship of volatiles production to biomass was done by Lappalainen et al. (1997). They found that measured total VOCs production per unit $CO_2$ evolved on a natural substrate (wood

shavings) from four species of mold averaged within the same order of magnitude. Two of the species tested, *Paecilomyces variottii* and *Wallemia sebi* are building-associated fungi.

**Table 2**. Analysis of volatiles produced by *Paecilomyces variottii* grown on foam insulation cytotoxic to HeLa cells. [1]

| | |
|---|---|
| ethanol | 3- methyl 1 butanol |
| n-methyl 2- propanamine | 4- methyl 1 hexane |
| furan | hexanol |
| 2- methyl furan | nonane |
| benzene | 2- hepatone |
| 2- methyl 1-propanol | hexanol |
| 2- butanone | decane |
| 1- butanol | hexanoic acid |
| 2- ethyl butanol | octanoic acid |
| octane | |
| methyl benzene | |
| 1,4 dimethyl benzene | |

1. *P. variotti was grown in all glass still culture fermentation systems with fitted ground glass joints (2 x 2,800 ml). After 10 days growth at 28 C on 500 ml of a glucose, salts, peptone/yeast extract/malt extract medium (CZ-met, Miller et al. 1991), headspace gases were collected using ultrapure compressed air on to Tenax GC. Volatiles from these four taxa were determined after thermal desorption on a high-resolution mass spectrometer (Finnigan MAT fitted with a Chemical Data System 320 thermal desorber).*

*In separate runs, volatiles were produced by culturing P. variotti on irradiated foam insulation soaked overnight in sterile wood extract. The wood extract was prepared by placing 125 g softwood shavings i h 2.5 had been soaked overnight. Pieces of the foam were placed in wire mesh baskets and then into*

*incubation flasks connected together by all glass connections. An agar slant containing a 10-day-old culture was macerated in sterile distilled water and added to CZ- met medium at 2.5% (v/v), which were incubated for 3 days on a rotary shaker. The resulting mycelia were centrifuged and resuspended in sterile distilled water. Approximately 35 mg dry weight cell suspension was added to each of the foam blocks. Cultures were incubated at 25 C and at days 9, 14 and 21 a glass tube containing 1 g activated Tenax GC inserted into the fermentation system and flushed with ultrapure air for 20 h. 15 mL DMSO was used to wash volatiles off the Tenax. This was diluted to 0.5, 0.25, 0.125 and 0.0625 and plus pure DMSO and added to microwells contain 24 h cultures of HeLa cells such that the amount of pure DMSO caused no changes in viability or colony forming ability. Significant (p= 0.01) depressions were seen in the numbers of viable cells at day 14 (by dye exclusion) and colony forming ability at day 21 in a dose dependent fashion. Volatiles collected the same way from uninoculated insulation and from a strain of Trichoderma viride were not toxic under the conditions tested. Volatiles collected from the liquid media as above also resulted in significant depressions in the numbers of viable cells at day 14 (by dye exclusion) and colony forming ability*.

Some information on the question of volatiles production in relation to biomass can also be gleaned from experiments on their biological activity. *Trichoderma viride* and *T. pseudokoningii* volatiles reduced the growth rate of some wood rot fungi by ca. 80% (Bruce et al. 1984). Growth inhibition of four species of wood rot fungi by volatiles of *T. aureoviride* was seen from the earliest stages of the experiments (Bruce et al. 1996).

Many experiments done on volatiles production by mold fungi have largely been uncritical in the sense that they involved the use of less sensitive methods of chemical analysis than needed and did not include a means to normalize the data per unit of fungal biomass such as $CO_2$ or mycelial dry weight. A further complication in interpreting many experiments on VOC yields is that the mycelia retain volatile compounds for long periods. Analysis of total VOC yields from the fungi involved in short-term in vitro experiments would therefore have to also consider volatiles from pentane extracts of the mycelia. If modest headspace volumes restrict volatile production because of end-product inhibition, further limitations are imposed on the data. Finally, the sensitivity of detection methods greatly impinges on whether a

compound will be detected when present or not. Some or all of these factors have led to some unrealistic interpretations of in vitro data.

Consonant with expectations based on the biochemistry of metabolite production, the weight of evidence from critical experiments is that both on natural and laboratory media, fungal volatiles are produced proportional to the number of cells in roughly similar amounts. This is based on chemical and biological activity analysis from several independent research groups with several taxa important in the indoor environment. Critical experiments have also demonstrated, as expected from the biochemistry involved, that volatiles produced are similar from growth on semi-defined media, a building material and from grain.

While the total amounts of volatiles produced is similar between different species of mold fungi, there are considerable differences between the individual volatiles produced between different species (Ammann 1999; Anon. 1986; Borjesson et al. 1979; Bruce et al. 1996; Halim et al. 1975; Kaminski et al. 1974; Larsen & Frisvad 1995a; Saito et al. 1979; Sunneson et al. 1995; Wheatly et al. 1997; Wilkins et al. 1999). Even within *Penicillium* species, these differences are sufficient to be detected using software that identifies patterns of data (Larsen and Frisvad 1995b). Analyses of volatiles produced by *Paecilomyces variotti* and *Trichoderma viride* were different using cluster analysis (using SYSTAT, Miller, unpublished data).

The approach of using metabolite pattern analysis for discrimination of bacterial and fungal populations and growth is an active area of research. Lipid signature profiles can be used for studying natural bacterial populations by biochemical means. It is based on the fact that for genera and some species within genera, there are characteristic patterns of cell lipids (White 1988). Air samples collected on microporous filters have been extracted with chloroform/methanol/water and analyzed by GC-MS for signature lipids followed by statistical treatments of the data to convert the analytical results to

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species profiles. The analysis provides information of the putative identities and biomass of dominant bacteria present in an air sample. Preliminary studies have shown that the lipid signature approach for bacteria gives values of "2-3 orders of magnitude" greater bacterial burdens than that estimated by cultural techniques (MacNaughton et al. 1997). This accords with the experience described in the literature that culturable estimates of bacteria in indoor air represent less than 1% of the total (Flannigan et al. 1991).

White et al (1980) used this approach for fungi in aquatic biofilms.  Additionally, based on 21 known lipids from mycelial extracts of four fungi (Miller et al. 1984), it was possible to separate the Ascomycete (*Leptosphaeria oraemaris)* from the three Deuteromycetes and *Dendriephiella salina* from *Sphaerulina oraemaris*  and *Monodictys pelagica* using cluster analysis (Miller 1999).  Current studies (JDM) are underway to determine if this approach would be useful for fungi in the indoor environment.

This demonstrates that successful, practical approaches exist to process analytical data from fungal metabolites present in air samples that can definitely be applied to fungal volatiles.

## 3.1.3  TOXICITY OF FUNGAL VOLATILES

It is unambiguously known that inhalation exposure to fungal spores and mycelial fragments can result in health effects of various kinds (e.g. Day 1996; Flannigan & Miller, 1994; Rylander & Etzel 1999; Verhoeff & Burge 1998). It is difficult to contemplate situations where people would be exposed to fungal volatiles in the absence of exposure to spores. Nonetheless, fungal volatiles have been suggested as possible contributors to adverse health effects (Sorenson 1989; 1990; Flannigan et al. 1991). It is said that fungal volatiles affect health by causing nasal irritation (Strom et al. 1990).

While the toxicity of fungal volatiles based on individual $LD_{50}$ values in animal studies is generally low (Sorenson 1989; 1990), this does not address the toxicity of the mixtures that are known. It is unambiguously known that the volatiles of *Trichoderma viride* and related species are highly toxic to other fungi (see above). In general, compounds that are toxic to fungi are also highly toxic to humans and animals. The volatiles of *Paecilomyces variotti* collected after 14 days were found to be highly cytotoxic to cultured human cells. Volatiles collected before and after this time were not or less toxic (Anon. 1986, Wiles 1986; Table 2). This makes sense in terms of the slowing down of active growth and hence volatiles production.  In view of the extremely small mass of chemicals comprising the mixture of *Paecilomyces* volatiles collected, its potency must be regarded as very high.

Volatiles from unnamed *Penicillium* species and *Trichoderma viride* affected ciliary beat frequency in animal explants.  In addition, these experiments showed that inflammatory mediators were released by the cells (Joki et al. 1993). All of these data are suggestive that where fungal growth is large in an enclosed space, the volatiles probably do contribute in some way to the nature of the complaints and or the objective health effects.

Miller et al. (1988) found that putative fungal volatile concentrations were an appreciable fraction of the total volatile organic compound burden in residential housing albeit when the amount of fungal growth was essentially visible. Strom et al. (1994) believed that fungal volatiles could pass through vapor barriers. Hence volatiles may explain occupant complaints under some circumstances in the absence of meaningful concentrations of spores in the space.

A second, possibly important consequence of the frequent (but not always) concurrent presence of fungal spores and fungal volatiles is that the odors to which humans appear

exquisitely sensitive, may affect trigeminal nerve-mediated effects (Ammann 1999; Strom et al. 1990).  These effects are the basis of sensory irritation and are a plausible mechanism for causing upper respiratory symptoms.  This is a controversial area of medical research and no stronger conclusions can be effectively drawn (see Ammann 1999).

## 3.2    PART II: BUILDING STUDIES

### 3.2.1   HISTORY OF MVOC STUDIES

Aromas and odors have been associated with mold growth undoubtedly since prehistoric times.  Mention of fungal odors has also long been included in some taxonomic descriptions of fungi (Coker and Beers 1943, Raper and Thom 1949, Hunt 1956).  These were subjective observations and not supported by any chemical analyses.  Yet they emphasize that odors from fungi are noticeable and some odors are specific enough to aid in the characterization of certain fungi.

Although there were earlier studies, more experimental work on fungi and volatile compounds began to be published in the 1960s.  Birkenshaw (1965) noted several volatile compounds that were produced by fungi and their associated metabolic pathways.  The production of fungal volatiles from wood staining fungi (Collins and Kalnins 1966; Collins 1976; Hubball and Collins 1978; Sprecher and Hanssen 1983) and black yeasts (DeHoag and Roeymans 1979) was studied.  Some fungi were also shown experimentally to respond to volatile compounds, and others to release volatiles, which attracted insects that could spread spores to new areas (Dick and Hutchinson 1966; Hutchinson 1971; DeGroot 1972).  The flavor characteristics of mushrooms and food processing molds that were due to volatiles also attracted attention during this time (Cronin and Ward 1971; Dwivedi and Kinsella 1974).  Work on fungal volatiles was expanding rapidly by this time and practical applications were envisioned.

Mold volatiles were explored in the 1970s as a means of detecting mold growth in stored grain (Kaminski 1972; Kaminski 1974), an effort that continued into the 1980s (Abramson et al. 1980; Seifert and King 1982). Also in the 1980s came the first applications of mold volatiles to the building sciences. A brief report from an indoor air conference in 1984 mentioned the odorous nature of some building molds from homes with moisture problems (Hyppel 1984). Samson (1985) noted that some occupants of damp buildings associated moldy odors with their symptoms and that this area deserved further research. Tobin et al. (1987) also indicated that some occupants respond to MVOCs with stuffiness or even wheezing. The possibility of health effects due to exposure to MVOCs was also discussed by Bissett (1987). Actual MVOC measures in a systematic study of mold in buildings were first reported by Miller et al. (1988). Since then, a number of investigators have applied MVOC analysis to the study of mold in buildings. Although the technique has proven useful in some circumstances, as outlined below, there are likely significant gains to be realized in its application to detecting mold in buildings. More recently, general reviews of MVOCs have been presented by Batterman (1995) and Ammann (1999).

## 3.2.2  MVOCs FROM GROWTH ON BUILDING MATERIALS

Several studies have focused on MVOC emissions from building materials. As expected, a number of compounds have been detected, including many that are prevalent in mold damaged buildings.

MVOC emission patterns from *Chaetomium globosum* were shown by Bjurman and Kristensson (1992) to vary with the composition of the artificial media. This emphasizes the importance of using realistic culture conditions such as culturing the fungi on the actual building materials (see Part I). Bjurman (1993) also cultured *Penicillium brevicompactum* and *C. globosum* on insulation materials. Uninoculated insulation materials were monitored. The uninoculated material did emit VOCs. However, the

emission patterns from the inoculated material included several compounds that were known irritants, and likely could cause typical moldy building related symptoms, including eye and upper airways irritation.

Korpi et al. (1998) used mixed cultures of molds and an actinomycete on building material samples to evaluate MVOC emissions. Microbial activity was verified by monitoring $CO_2$ production. No single MVOC was sufficient as an indicator of mold growth. This is not surprising since there is a substantial variety among MVOC patterns, and this complexity would be expected to increase when mixtures of microbes are used. Again, the emission of some MVOC compounds from uninoculated materials was noted. This background level emphasizes the need for careful collection of control samples in building investigations. The complex patterns noted also emphasize the need for additional detailed analysis of the MVOC fingerprints from these molds.

### 3.2.3 STUDIES MEASURING MVOCS IN BUILDINGS: OCCUPIED SPACES

Hyppel (1984) found that half of the isolates recovered from a study of building molds had a detectable odor and that one in four had a characteristic mold odor. Volatiles collected from one isolate were analyzed chromatographically. The odor contributing to the building related problem was attributed to geosmin. This study did plausibly link mold odors with complaints from buildings with moisture problems, although the samples in this study were taken from laboratory cultures rather than from the buildings. For reasons outlined in Part I, it is not possible to evaluate the claim that half of the isolates recovered did not produce strong odors. The process of isolation can affect metabolite production in cultured strains. Additionally, fungi in damp buildings never grow alone, i.e. usually there are several species growing.

To our knowledge, the first report of MVOC samples that were taken in field studies was from a survey of 52 houses across Canada in 1986 (Miller et al. 1988). In about 35

houses, an occupant had health complaints thought to be related to the house. Several measures were used to survey these houses for evidence of fungal growth. These included culturable fungi from air, culturable fungi from dust, ergosterol levels in dust, and MVOCs from air.

Based on culture studies (described in Part I), three fungal volatiles were considered most likely to only come from microbial sources: 3-methyl-1-butanol, 2-hexanone, and 2-heptanone. Regardless, these MVOCs were present in most of the houses. Where these MVOCs were elevated, there was not a strong correlation with the other indicators of fungal growth. These authors concluded that elevated MVOC levels only detected serious cases of mold growth and that other measures of mold growth were perhaps more reliable.

To date, there have been no larger surveys of buildings than the Canadian study cited above. There have been other studies though, usually directed at more specific features of mold growth in buildings. McJilton et al. (1990) established that in three buildings with serious odor problems, a bacterial source of the odor was likely. In all three cases, air conditioning condensate contained growth of an unknown red bacterium (subsequently identified as *Methylobacterium organophilum* Subgroup llb; Steve Reynolds, personal communication) in the complaint, but not the non-complaint areas of the building. Air samples taken in the complaint areas of the buildings and from culture headspace samples all contained 1-butoxy-2-propanol, which has an offensive odor. Thus, a circumstantial case was built for the idea that bacteria were generating the odor.

Bjurman and Kristensson (1992) sampled the volatiles emitted from an isolate of *Aspergillus versicolor* recovered in a study of houses with mold problems. Although they did not include field samples from the buildings, this study was specifically designed to address the odor due to building molds. These authors identified ethylhexanol in the headspace of cultures of A. *versicolor*. This compound was also

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detected in samples from contaminated building materials from mold-contaminated houses (although the data were not presented). Due to these correlations and the pungent odor of ethylhexanol they concluded that this compound largely explained the complaint odor in the moldy houses they were investigating.

Strom et al. (1993; 1994) presented results from MVOC analysis of air in about 80 buildings. These included buildings with moisture problems, and reference buildings, as well as some outdoor control samples. These authors looked for MVOCs that were considered to indicate microbial growth (i.e. the least likely to have non-microbial sources; Table 3). Air samples were analyzed for compounds on this target list while operating the mass spectrometer with selective ion monitoring (SIM). It is important to note that fungi produce numerous VOCs, and many also have non-microbial sources. The compounds on this target list are those that are considered the most likely to be due to microbial sources. Significantly many of these compounds have very low odor thresholds (see Table 4). This follows the logic of Miller et al. (1988) in focusing on the three compounds mentioned in their study.

With this technique the MVOC levels in the problem buildings were higher than in the reference buildings or in the outdoor air samples. Unfortunately, Strom et al. presented rather limited mycological data on the buildings. Analyses for living spores (viable propagules) from bulk samples were reported from three buildings in the second study. The MVOC technique was shown to be capable of distinguishing between two classes of buildings. It is difficult to relate these buildings to moldy buildings in other studies.

The Strom et al. work presented data showing that plastic sheeting and other building materials are permeable to the MVOCs on their target list. This implies that mold odors can penetrate from an area with mold through vapor barriers into occupied spaces that are free of mold growth. As long as the integrity of the vapor barrier is maintained,

culture based air sampling might not detect spores.  Details were not presented on how

the test atmosphere was generated (i.e. on equal mass or equal vapor pressure basis),

| Table 3. Target microbial volatile organic compounds (MVOCs) cited in building studies that are considered useful indicators of microbial growth. | | | | | |
|---|---|---|---|---|---|
| | | | | ○: Class A compounds ★: Class B compounds | |
| Compound (CAS number) | Miller et al 1988 | Strom et al 1993 | Strom et al 1994 | Morey et al 1997 | Wessen et al 1995, 1996b |
| 3-methylfuran (930-27-8) | | ● | ● | ● | ○ |
| 3-methyl-1-butanol (123-51-3) | ● | ● | ● | ● | ○ |
| 3-methyl-2-butanol (598-75-4) | | ● | ● | ● | ○ |
| 2-pentanol (6032-29-7) | | ● | ● | ● | ○ |
| 2-hexanone (591-78-6) | ● | ● | ● | ● | ○ |
| 2-heptanone (110-43-0) | ● | ● | ● | ● | ○ |
| 2-octanone (111-13-7) | | ● | | | |
| 3-octanol (20296-29-1) | | ● | ● | ● | ○ |
| 1-octen-3-ol (3391-86-4) | | ● | ● | ● | ○ |
| 2-octen-1-ol (22104-78-5) | | ● | ● | ● | ○ |
| 2-methyl-iso-borneol (not available ) | | ● | ● | ● | |
| geosmin (19700-21-1) | | ● | ● | ● | ○ |
| 2-isopropyl-3-methoxy-pyrazine (25773-40-4) | | ● | ● | ● | |
| 2-methyl-1-propanol (78-83-1) | | | ● | | ○ |
| 1-butanol (71-36-3) | | | ● | | ○ |
| 3-octanone (106-68-3) | | | ● | ● | ○ |

| **Table 3.** Target microbial volatile organic compounds (MVOCs) cited in building studies that are considered useful indicators of microbial growth. | | | | | |
|---|---|---|---|---|---|
| | | | | **o**: Class A compounds <br> **★**: Class B compounds | |
| **Compound (CAS number)** | **Miller et al 1988** | **Strom et al 1993** | **Strom et al 1994** | **Morey et al 1997** | **Wessen et al 1995, 1996b** |
| Dimethyldisulfide 624-92-0) | | | | | **O** |
| Methylisobutyrate (547-63-7) | | | | | **★** |
| 2.6.6-trimethyl-bicyclo(3.1.1)heptan-3-one (not available) | | | | | **★** |
| 4-methy-1-isopropyl-3-cyclohexen-1-ol (not available) | | | | | **★** |
| 3.6.6-trimethyl-bicyclo(3.1.1)hept-3-en-2-one (not available) | | | | | **★** |
| 2-pentanone (107-87-9) | | | | | **★** |
| Endo-borneol (507-70-0) | | | | | **★** |
| Fenchon (1195-79-5) | | | | | **★** |
| Styrene (100-42-5) | | | | | **★** |

**O** class A compounds (initial list of useful marker MVOC compounds) as defined by Wessen et al, 1995b

**★** class B compounds (additional MVOCs not on the class A list) as defined by Wessen et al, 1995b


but there were large differences reported in the permeability of some of these compounds through the plastic. This phenomenon is important in occupied spaces from MVOC sources in interstitial spaces (building envelopes, subfloors, etc.). This, in turn, could alter any interpretation that is based on ratios of individual MVOCs.

Bayer and Crow (Bayer and Crow, 1993a; 1993b) reported MVOC analyses from several buildings, where concurrent MVOC and bioaerosol sampling were conducted. They also

examined MVOC emissions from certain of the fungi recovered from buildings. The mycological assessment, although presented in abbreviated form, indicated that the complaint areas of the building likely contained areas of mold growth. The principal VOCs recovered in their analyses were solvents such as ethanol and acetone, and they reported higher levels in the complaint areas. These authors concluded that portions of the VOC load in buildings may due to microbial activity, but were routinely attributed to other sources. A subsequent study in three schools by this group (Bayer et al. 1995) used a similar design. This study included chamber sampling from contaminated HVAC duct material though, and reached essentially similar conclusions. Most workers measuring MVOCs have considered these solvent compounds not to be useful, since they are emitted from biological and non-biological sources. Consequently, these studies cannot be easily compared to most of the other studies on MVOCs in buildings.

| Table 4. Threshhold Reference Odor Description[1] | | | | |
|---|---|---|---|---|
| | | Odor Threshhold µg/m³ | | See [2] |
| CAS # | Chemical Name | Min | Max | Odors |
| 123-51-3 | 3-Methylbutan-1-ol | 68.7 | 255.2 | |
| 591-78-6 | 2-Hexanone | 303.7 | 1557.5 | |
| 110-43-0 | 2-Heptanone | 435.9 | 1021.8 | |
| 111-13-7 | Hexyl acetate | 1658.2 | 1658.2 | |
| 3391-86-4 | 1-Octen-3-ol | | | Metallic, Cedarwood |
| 78-83-1 | 1-Methylpropan-1-ol | 276.5 | 59,1000. | |
| 71-36-3 | 1-Butanol | 448.4 | 7792.1 | Cresote; Hay |
| 106-68-3 | 3-Octanone | 316.0 | 316.0 | |
| 624-92-0 | Dimethyldisulfide | 13.7 | 226.9 | |
| 107-87-9 | 2-Pentanone | 1725.3 | 12499.0 | |
| 507-70-0 | Borneol | 13.2 | 13.2 | |

[1] Devos M., F. Patte J. Rouault, P. Laffort, and L.J. vanGemert. Standardized Human Olfactory Threshholds. New York: Oxford University Press, 1990.
[2] American Society for Testing and Materials. Atlas of Odor Character Profiles. ASTM Data Series DS 61, compiled by A. Dravnieks. Philadelphia: American Society for Testing and Materials, 1985.

| Table 4. Threshhold Reference Odor Description[1] | | | | |
|---|---|---|---|---|
| | | **Odor Threshhold** µg/m³ | | See [2] |
| 100-42-5 | Vinyl benzene | 223.6 | 1347.0 | |

Kumlin et al. (1995) presented case histories of buildings in which microbial odor problems occurred in this way. Mold growth in subfloor insulation over an on-grade concrete slab was fostered by condensation. The MVOCs that were produced by this growth penetrated the floor into the occupied space, and led to complaints. Again, there may have been too few spores escaping to permit culture based sampling to detect this growth, without some sort of destructive sampling of the flooring system.

A study by Wessen et al. (1995) further stressed the general principle that MVOCs can distinguish between problem and reference buildings. This paper reported on over 500 samples, mostly from Sweden, but including some from Germany. Unfortunately, there were no culture data presented to independently establish that their "problem" buildings had unusual microbial contamination.

In order to better detect problem buildings, Wessen et al. (1995) examined the variety of compounds emitted from laboratory studies of molds cultured on different building materials. Based on these results, additional compounds were included in the target list (Table 4). These were referred to as the method B compounds, or in a subsequent study, as the class B compounds (Wessen and Schoeps 1996b). Overall, the ratio of the mean indoor concentration to the mean outdoor concentration was not affected by including the additional compounds. This means that the B group was not more prevalent indoors than the A group. However, seven specific cases were presented where the additional compounds were collectively the major portion of the MVOCs in the sample. In these cases, including the additional compounds would aid in the interpretation. It is difficult to reliably judge from this limited number of cases the degree of benefit from this expanded target.

A larger set (2200) of samples was used to evaluate certain indicator ratios of MVOCs (Wessen and Schoeps 1996a). This effort was directed at improving interpretations from MVOC samples taken from "sick" buildings contaminated building materials and pure cultures of molds from buildings. Three indicator MVOCs were considered, dimethyl disulfide, 3-methylfuran and 1-octen-3-ol. When a building problem is due to sewer gas intrusion, a high relative proportion of the MVOCs are sulfur compounds, which are responsible for the typical sewer smell.

Conversely, 3-methylfuran and 1-octen-3-ol were claimed to be typical of mold growth rather than sewer gas, so mold growth should be sought if these dominate. Further, the adsorption affinity of 3-methylfuran is much lower than 1-octen-3-ol. These authors suggest that 3-methylfuran will rapidly dissipate due to its low adsorption affinity, and hence significant levels of 3-methylfuran would only occur where mold growth is still active. Samples with 1-octen-3-ol greatly in excess of 3-methylfuran should thus suggest mold growth that is no longer active. It is important to note that the concentration of 3-methylfuran was lowest or second lowest of all MVOCs in the case studies presented by Strom et al. (1993; 1994). This was also in true laboratory cultures (Wessen and Schoeps 1996b). This suggests that even when present, this compound may be difficult to recover. Although 3-methylfuran may be a useful indicator when present, additional information is needed on emission patterns of 3-methylfuran to preclude over- interpreting its absence.

Smedje et al. (1996) used MVOC analysis in a survey of 38 schools in Uppsala, Sweden. This included symptom comparisons collected by questionnaire, and exposure measurements that included MVOC analyses and total and culturable airborne mold concentrations, as well as 12 other factors. In a step-wise regression analysis, several factors were significantly related to reported asthma: atopy, stress, recent indoor painting, and the school exposure factors total (but not viable) molds, and

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certain MVOCs.  These MVOCs were 2-methyl-iso-borneol, 3-methylfuran, 2-heptanone, and 1-octen-3-ol.  These results suggested that mold exposures were important risk factors for asthma (this is consonant with many studies, see Part 1). Surprisingly, exposure factors that are known to be important, such as pet allergen, were considered but not found to be significant in this study. This limits the usefulness of this report.

MVOC sampling in buildings has a significant advantage over traditional bioaerosol sampling methods.  MVOC sampling is usually conducted over several hours whereas spore sampling is typically for only a few minutes.  Since bioaerosol levels in air fluctuate considerable, MVOC samples may be less affected by this, at least in short-term samples.

An advantage of MVOC sampling over bioaerosol sampling is that the MVOC sampling devices are rather small.  This means that they can be easily be placed in interstitial spaces in structural elements, such as walls, ceilings, and flooring assemblies, which sometimes are the site of the microbial growth.  This is a major advantage since most other types of bioaerosol samplers are too large to fit into these spaces.  This advantage was used by Wilkins et al (1999) to improve the sensitivity of the method by sampling air from interstitial spaces, where the MVOC concentrations were higher due to proximity to the microbial growth.  Such relatively non-destructive sampling also can help locate the source as well as confirm the presence of microbial growth in a complaint area suspected of having microbial growth.

Some studies are also available that have focused on special use buildings, special sources in buildings, or on exposures due to special procedures in buildings.   Wilkins and Larsen (1995) sampled headspace volatiles from decomposing household waste.  It certainly should not be surprising that MVOCs were detected in this case.  However, this underscores the value of careful inspections in building investigations to eliminate,

or account for, unexpected sources of pollutants.  Similarly, Norback et al (1995) documented exposures to MVOCs that occurred during painting operations.  These resulted from using latex paints that were spoiled by bacterial growth.  Again, the presence of MVOCs should be expected, but sampling in these circumstances might wrongly implicate microbial growth in the building if inspections and interviews were not thoughtfully conducted.

Morey et al. (1997) showed that MVOC sampling and data interpretation both require care.  In one large, former industrial building where partially rotted timbers were retained as part of a historic restoration, control (outdoor, rooftop) air samples were heavily contaminated by the same MVOCs as found indoors.  Concurrent outdoor control samples taken on the roof of nearby building contained negligible amounts of MVOCs.  The rooftop MVOC samples from the contaminated building were taken near a rooftop access door.  These authors considered it likely that the MVOCs detected in the rooftop sample at this building were escaping from the building via the access door and contaminating the control.  Although the MVOC levels in this building ($mg/m^3$ levels) are not commonly seen, this case illustrates the care that should be taken with control samples.  With cases from other buildings, Morey et al. (1997) showed that MVOC sampling could sometimes detect microbial growth where culture results were typical of a non-problem building.  The interpretation of these MVOC data required care, however, since some suggested mold growth only when the presence of individual MVOCs was considered.

New data from a building undergoing microbial remediation confirms the work of Strom et al (1993) that MVOCs diffuse through the whole building while fungal spores were prevented from moving by containment barriers (plastic sheeting; Morey et al. 2000).  The wet basement of this building was contaminated by growth of several Aspergillus species (including *A. versicolor*) concentrations of about $10^2$ to $10^3$ cfu/$m^3$.  Other portions of the building, which had been separated from the basement by polyethylene

sheeting, were dominated by phylloplane fungi such as *Cladosporium cladosporioides*. MVOC concentrations throughout the building (both inside and external to the containment barriers) generally exceeded 100 μg/m$^3$.   MVOC sampling in this case showed that the moisture problem had not been fixed and more importantly, that sampling any one part of the building (even remote from the barrier) could identify the existence of a water problem.

## 3.2.4  HVAC SYSTEM FILTERS

Although building investigations with MVOC sampling have sometimes included samples from within the HVAC, there are some studies that have focused specifically on HVAC filters and insulation.  These have implicated microbial growth on filters as possible sources of MVOCs in buildings.  It is very difficult though to distinguish between MVOCs emitted as a result of microbial growth and being re-emitted after adsorbing compounds from the air stream.

Pasanen et al. (1990) detected short chain fatty acids and aldehydes from used filter material that was incubated in humid (75% RH) chambers for 7 days.  As from any used filter, fungi and bacteria were common.  They monitored $CO_2$ emission rates though as an indicator of microbial activity, and this correlated generally with the emission of volatile fatty acids and aldehydes (Pasanen 1990; Martikainen 1990).  This is consistent with a microbial source of these compounds, but surprisingly, other VOCs typically seen with mold growth were not reported in the air sampled from these chambers.

MVOCs (aldehydes and ketones) from mold growth in HVAC filters have been implicated in occupant complaints.  Extensive sampling in the airstream before and after the filters, and in laboratory incubations, was used to show that mold growth was a likely source of the compounds (Schleibinger 1995; 1996).  These studies presented evidence that a portion of the VOC load in a building likely derives from microbial growth in HVAC filters, although sufficient levels to account for occupant complaints were not seen.  These studies do underscore the need for diligent maintenance of air-handling units (AHUs).  It was surprising that these authors did not see many of the compounds reported in other field studies in mold-contaminated buildings.  This may be due to differences either in sampling or in analysis methodology.  In a further study by this group filter materials were incubated under controlled humidities, and the same compounds were reported (Schleibinger et al. 1997).

Insulating material designed for use in HVAC systems was used as a substrate for mold culture in one study (Ezeonu 1994).  In this study, *Acremonium obclavatum* and *A. versicolor* were grown on insulation incubated at about 95% RH.  Under these conditions, which are common downstream of cooling/dehumidification coils in humid or even moderate climates, several MVOCs were detected from each mold on the insulation.  *A. versicolor* produced the irritant 2-ethyl hexanol.

## 3.2.5  OCCUPATIONAL ENVIRONMENTS

A recent series of papers by Fischer et al. (1998, 1999) document the special exposures to MVOCs that occur in a municipal waste composting facility.  The workers claimed to be able to detect specific patterns in the emissions from a number of the species recovered from the compost.  Specific molds were also provisionally identified as the source of some MVOCs.  These studies also detected seasonal shifts in both the mix of prevalent species and the MVOCs present.  These studies are some of the most detailed analyses reported to date on MVOC production in occupational environments.  These authors concluded that additional work is needed concerning the specific profiles of MVOCs from the important species in order to make this technology useful.

## 3.2.6  DUST SAMPLES

The analysis of settled dust from buildings has proven useful in many studies for assessing either culturable fungi, allergens or endotoxin.  VOC emissions have also been examined from collected dust.  This may be useful in building studies.  Wolkoff and Wilkins (1994) showed that a variety of VOCs are released from dust when heated to $120^{\circ}C$.  Many of these compounds were aldehydes and carboxylic acids, some of which may result from microbial growth.  Hirvonen et al. (1994) also analyzed VOC emissions from dust heated from $50\text{-}150^{\circ}C$. They found compounds that were likely microbial oxidation products.  Dust samples from different buildings gave similar

results, but there was no selection of problem or complaint buildings in this study. Clearly, though, it is possible to sample and identify VOCs from dust collected in buildings. This may be useful as an indicator of past conditions in a building if indicator MVOCs were detected.

MVOCs were produced from artificial inoculations of sterilized house dust with *A. versicolor* (Pasanen 1996) and incubation of non-sterilized dust (Korpi 1997). Pasanen et al. (1996) showed that *A. versicolor* could grow in sterilized dust and emit MVOCs. Although not surprising, this experiment did show that compounds considered indicators of mold growth were produced under realistic conditions. In the second study, eight compounds comprised the major portion of the MVOCs released. Among these eight, three are used to indicate microbial growth, including 2-heptanone, 2-hexanone and 2-methylfuran. Other compounds, including 3-octanone, 1-octen-3-ol and 3-methyl -1-butanol were detected as well from the incubated dust. Note, however, that in these studies, dust was used as a growth medium rather than as a sampling medium. Regardless, dust is an important component of buildings. When molds grow in building dust, some of the MVOCs that are released are useful indicators of microbial growth.

Korpi et al (1997) also found that MVOC emissions were higher from samples of building dust incubated at a relative humidity of 85% than samples incubated at 95%. This was contrary to other indicators of microbial activity, including $CO_2$ generation and increases in colony forming units. These authors cited the established problems of high humidity interfering with commonly used sorbents. Since high RH occurs in building systems that need to be sampled for MVOCs, there is a need for improved sampling technology to avoid this problem.

Wilkins et al. (1997) compared the VOC emission patterns from building dust that was collected in rooms that were visibly moldy, and in control (non-moldy) rooms. This analysis was coupled with a standard analysis for cultural fungi, which showed that the

dust from the moldy rooms was dominated by *Penicillium commune*, *P. chrysogenum* and *A. versicolor*. Rather than looking for certain indicator compounds, these authors statistically analyzed the emission patterns using principal components analysis (PCA). This technique was able to distinguish between the dust from moldy rooms and from control rooms. This indicated the potential that may be realized from MVOC sampling as more detailed analysis techniques, such as PCA are applied to these data sets.

Substantial additional work is required before reliable interpretations will be possible from dust MVOC analyses. Dust represents a historical record of the building environment though, and may be the only type of sample that can indicate past conditions. Since historical information on conditions would often be useful in building investigations, reliable interpretation of MVOCs from dust samples would prove very valuable.

### 3.2.7 INTERPRETATION OF FIELD DATA

Currently, most field investigators interpret MVOC data by visually comparing the compounds found and their concentrations among sampling sites. As discussed above, there are limitations to the technique. However, as many cases described above indicate, MVOC sampling sometimes provides an indication of mold growth in buildings where it is undetected by culture sampling.

It can be helpful to discriminate between fresh mold growth and mold growth that had developed over longer periods of time. The production of MVOCs (as described above) is a function of biomass, but the adsorption and retention by building materials may also provide useful information. Some authors assert that the differential adsorption of MVOCs (and subsequent desorption) may suggest one or the other case. It has been shown that grain exposed to MVOCs adsorbed greater amounts of 1-octen-3-ol and 3-methyl-1-butanol than methylfuran (Borjesson et al. 1994). Since methylfuran is not

readily absorbed, it should only be detected when it is being produced, i.e. indicating fresh, or current growth (Wessen et al. 1996a). Conversely, in a building with a past occurrence of mold growth, 1-octen-3-ol and 3-methyl-1-butanol might persist as an indicator of the growth, but without the presence of methylfuran. In practice though, the recovery of methylfuran was among the three lowest of 12 compounds tested (Wessen et al. 1996b). Hence, the absence of methyfuran should not be over-interpreted.

Similarly, since dimethyldisulfide (DMDS) is a dominant component of sewer gas, buildings infiltrated by sewer gas have appreciable amounts of DMDS. If DMDS is included in the list of target compounds, then a DMDS concentration equivalent to the concentration of all other MVOCs in a sample has been suggested as supporting the specific interpretation of sewer gas infiltration (Wessen et al. 1996a).

## 3.3    ANALYTICAL METHODS

All investigators use variants of similar methods using gas chromatography and mass spectroscopy (GC/MS) analysis of samples collected on adsorbents typically with thermal desorption. These reports seldom report recovery data for the dominant compounds and include analytical error. Miller et al. in two reports (Anon. 1986; Venayak et al. 1987) tested the recoveries of 10 volatiles produced by molds. Aliquots of these were placed in all glass chambers, allowed to evaporate, and ultra zero air passed through for 4 hours. Volatiles were collected on cold traps at -78 and -196 C, respectively and in a separate experiment using a series of GC column materials. All the volatiles were collected at -78 C and Tenax GC was found to recover the test volatiles in similar fashion to the -78 C cold trap. In such case, Miller et al. (1988) undertook the field study with knowledge of the specific analytical performance of the target volatiles and the sample collection method.

As noted above, the collection of MVOCs in areas with high relative humidity is an unexplored problem as indeed is the effect of building material RH. Many building materials emit higher concentrations of chemical VOCs when they are wet.

## 3.4    SUMMARY MVOC DATA FROM FIELD SAMPLES

Summary data from 668 field samples, mostly from the United States, are presented in Table 5 and in Figures 2 and 3.  Where possible, results from outdoor samples were categorized separately.  This permits some comparison with other published data, and discussion of interpretation of field data.

**Table 5**. Descriptive parameters of a MVOC results from 668 air samples submitted from field investigations.   (Unpublished data)

| | $n^1$ | $nd^2$ | Total MVOC ($ng/m^3$) | | Compounds / sample |
|---|---|---|---|---|---|
| | | | Average | Median | |
| All Samples | 668 | 12.4% | 25008 | 2362 | 2.68 |
| Outdoor Samples | 91 | 38.5% | 2358 | 334 | 1.31 |
| Indoor Samples | 577 | 8.0% | 28578 | 2945 | 2.89 |
| I/O ratio | | | 12.1 | 8.8 | |

[1] number of samples
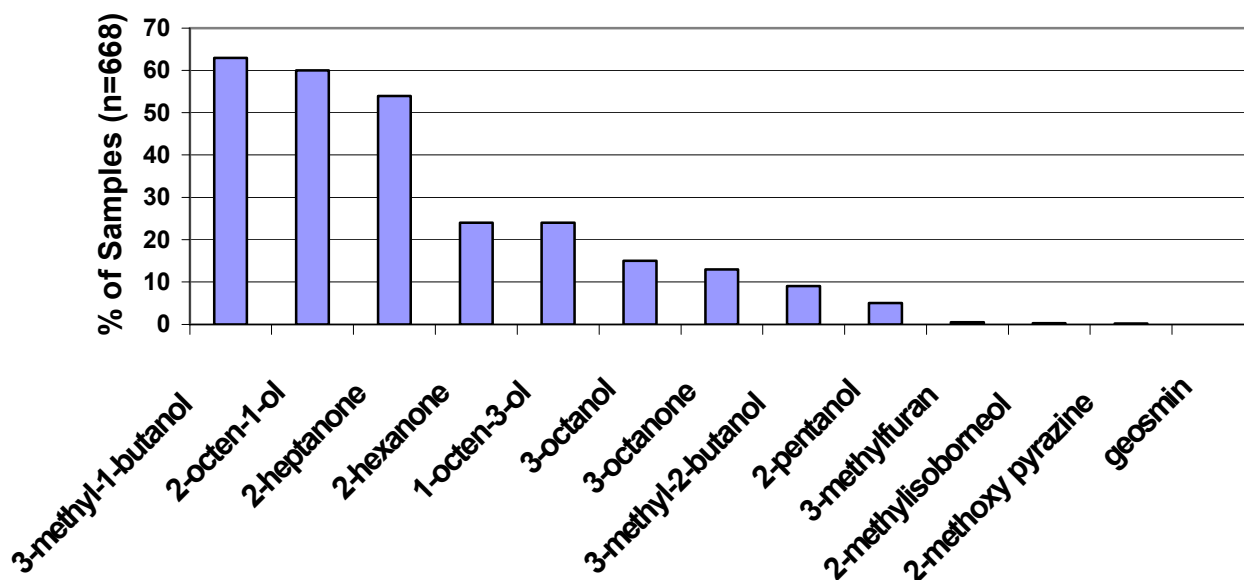[2] nd =non-detect (no target MVOCs detected in the sample)

**Figure 2**. Prevalence (%) of thirteen individual microbial volatile organic compounds (MVOCs) in a sample of 668 field samples. These were predominantly from commercial buildings in the United States. (Unpublished data)

These data contain two biases. First, since MVOC sampling is most often used in investigations of complaint buildings, presumably most of the samples are from complaint buildings where mold growth was at least suspected. So these data apply to complaint buildings rather than buildings in general.

Second, samples were assigned to the "outdoor" category only if the sample was clearly identified as such. For various reasons, though, investigators may not reveal sample location. So, likely some outdoor samples are retained in the "indoor" category.

Furthermore, it is often not possible to separate samples from problem (or complaint)

areas and control (or non-complaint) areas.  So, undoubtedly some samples in the

indoor category contained minimal or no MVOCs since they were from areas without

mold growth, or were from outdoors.  This bias will tend to lower the summary MVOC

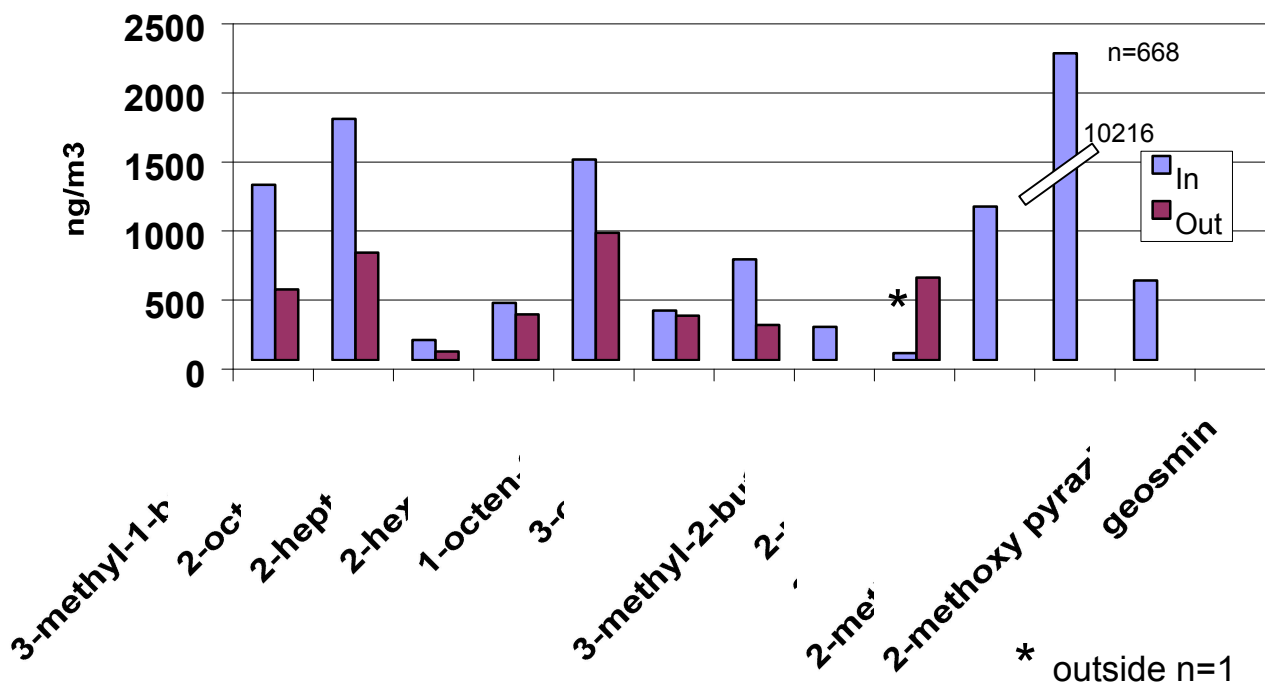values of the "indoor" group and make the results more conservative.



Figure 3. *The median values of thirteen individual microbial volatile organic compounds (MVOCs) in a sample of 668 field samples.  Outdoor samples were those that were identified by the investigator, all others were considered indoor samples, including those with no location.  Complaint and control locations were not provided often enough to permit separation of these groups.  (Unpublished data)*

The prevalence of 13 individual MVOCs among 668 samples submitted for analysis are

graphed in Figure 2.  One sees from these results that MVOC prevalence varies greatly.

 Three compounds are in at least 50% of all samples, while four were detected in less

than 1%. The prevalence of the other six varied from about 5 to 25%. To our knowledge, only two published reports mention prevalence of individual compounds among field samples. Miller et al (1988) detected 3-methyl-1-butanol, 2-heptanone and 2-hexanone in 44%, 89% and 89% of houses in that study, and considered non-microbial sources to be unlikely. These are three of the four most prevalent compounds listed in Figure 2. Wessen and Schoeps (1996a), on the other hand, reported that among 2200 samples from Swedish buildings, 3-methylfuran was present in 55% of samples and 1-octen-3-ol was in 40%. In comparison, 1-octen-3-ol occurred in about 25% of samples from the United States, but geosmin was not detected at all.

Median values for the concentrations of individual compounds are given in Figure 3. Among the more prevalent MVOCs, four have indoor median values above 500ng/m$^3$. These are 3-methyl-1-butanol, 2-octen-1-ol, 1-octen-3-ol and 3-octanone. The ratios of the median indoor and median outdoor concentrations for these four compounds were among the highest five such ratios of these compounds (Figure 4). This indicates that these compounds do distinguish between the problem buildings in this database and outdoor air.

Total (or sum) concentrations from indoor and outdoor samples have been averaged and reported. The indoor / outdoor ratio (I/O) was 13 from data in Wessen and Schoeps (1996b) for the list of compounds comparable to the United States database. In Wessen et al (1994), the I/O was 17 for buildings in Germany and 17.9 for Swedish buildings. The I/O in the database from the United States was 12.1 for the average of total MVOCs and 8.8 for the median totals (Table 3), which agrees well with the other two reports. These other two reports included only buildings that reportedly had microbial growth problems. Not surprisingly, these database summaries indicate that in buildings known or presumed to have problems of microbial growth, there are elevated levels of certain marker MVOCs.

It can also be seen in the United States database that indoor samples from (presumably) moldy buildings have more compounds than outdoor samples. On average, outdoor samples had 1.3 compounds per sample, but the average indoor sample had 2.9 compounds. The variety of compounds may be a feature that deserves further attention in the interpretation of data from building MVOC samples. To our knowledge, there are no published compound/sample data for comparison.
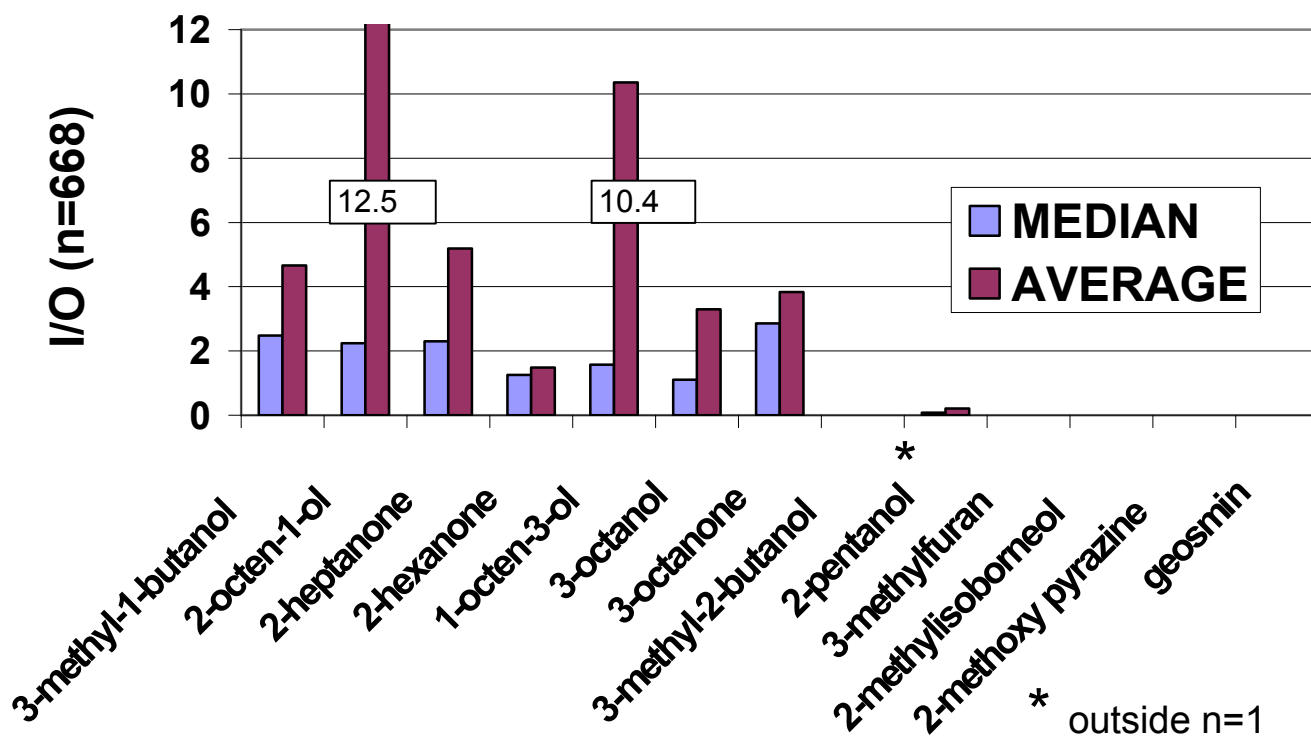


**Figure 4**. *Ratio of indoor to outdoor median (and average) for each of thirteen individual microbial volatile organic compounds (MVOCs) in a sample of 668 field samples.    (Unpublished data)*

## 3.5    CONCLUSIONS

MVOC production and release are inherent parts of mold growth in or on building materials. This often is the key feature that draws attention to mold growth problems in

buildings.  Although direct human health effects due to MVOC exposure are unproven at this time, the objectionable odors and low odor threshold of some MVOCS can readily cause occupant complaints and often lead to building investigations.

The available evidence indicates that specific compounds may be produced by numerous species of molds.  Species of molds have unique profiles of MVOCs, though, especially when compounds that are minor components of the profile are examined. Statistical pattern analysis will aid the characterization of these patterns.  Detecting these profiles will require detailed analyses though with samples collected from carefully controlled growth cultures.  The required technology to do this is available and established.  This technology needs to be applied to a more useful selection of building molds since there is no detailed MVOC information available for many of the common building molds.

Once developed, this ability has the promise of aiding building mangers and owners with a direct, objective tool to confirm the presence of mold growth and perhaps even indicate the mold types that are involved.  Perhaps most importantly, the ability to sample MVOCs from a building and analyze the patterns of MVOCs may prove useful in routinely assessing or monitoring the performance of a building with regard to moisture issues.

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## 4.0    PHASE I OBJECTIVES

Task 2 of 1072-TRP included 2 objectives.  These objectives were to prepare a work plan for Phase II and to prepare a report of Phase I work.   The work plan that was prepared for Phase II is in the following section.  This document, including the literature review and the work plan are the report for Phase I.

## 4.1    PHASE II WORK PLAN PROJECT GOAL

The goal of Phase II is to establish methodologies for the identification of MVOCs in the building environment in order to identify their role in poor indoor air quality and also to evaluate their interpretive utility in identifying mold contaminated building environments.  There are three components to this effort:

4.1.1   Identify and quantify specific MVOCs associated with specific organisms.

4.1.2   Determine MVOCs under realistic building conditions and establish sampling procedures.

4.1.3   Validate these methodologies in Real Building Environments.

Establishing and validating MVOC sampling and analysis methodologies for building environments is complex.  Identifying and validating remediation strategies for mold and/or odors arising from mold and evaluating appropriate technologies is beyond the scope of this work plan.

## 4.2    PHASE II WORK PLAN APPROACH

4.2.1    Identify important MVOCs.

4.2.1.1    Collect strains of species that have been repeatedly isolated from building materials.  Depending on the resources available, this might include *Chaetomium globosum*, *Penicillium viridicatum*, *Eurotium herbariorium*, *P. aurantiogriseum*, *P. citrinum*, *Stachybotrys chartarum*, *Aspergillus sydowii*, *P. commune*, *Eurotium repens*, *A. versicolor*, *Paecilomyces variotti* and *Cladosporium sphaerospermum*.

4.2.1.2    In all glass fermentation systems, produce large quantities (> 100mg) of volatiles from each strain collected on suitable adsorbents.  Use gas chromatographic mass spectrometry (GC/MS) to identify and quantify the compounds collected.  The intent is to expand the list of known and usable compounds produced by the target species from 8 -10 per species to 30 -50.

4.2.1.3    Based on this knowledge, use pattern analysis and predictive statistics to determine which compounds have the best potential to separate species.  However, because absolute identification for the many compounds may be difficult, pattern analysis should also be performed using recent chemometric software techniques that include all mass spectra collected over the entire chromatographic run.  Once these patterns are found, identification of the individual responsible compounds may be possible.

4.2.2    Determine MVOCs under realistic building conditions and establish sampling procedures.

4.2.2.1    Select representative species from Task 1 to grow on appropriately conditioned substrate at a volume loading and air exchange rate that would be representative of a building environment.  Collect emissions on sorbent and analyze using GC/MS as before.  Establish detection limits for MVOCs spanning

the volatility range.  If certain important MVOCs cannot be observed (for example, the fugitive 3-methylfuran or low-vapor pressure geosmin), increase the loading (perhaps by reducing the volume of the chamber) and decrease the air exchange rate until they can be detected.

Due to the lack of sunlight, hydroxyl radical, and typical reactive organics in the chamber environment, adjust anticipated MVOC decay rates for atmospheric oxidation using accepted estimation techniques.

4.2.2.2      Based on these findings, establish sampling procedures for building environments.  This would take factors into account such as ventilation of the building and dilution volumes of regions sampled.  For example, in heating ducts, determine whether it would be necessary to close off the vents 24 hours in advance before sampling, or what air-flow through the ducts would be unacceptable for detecting MVOCs even if molds were present.

4.2.3   Conduct field validation studies

Identify at least 3 problem odor environments with contaminated ventilation systems or rooms (particularly those with sources of moisture vapor) and determine the source of the contamination, the identification of the organisms, the identification of the MVOCs, and levels of both in the environment.

## 4.3    PHASE II SCHEDULE FOR COMPLETION

The following table contains a proposed schedule for addressing the Tasks recommended for Phase II.  The time required for completing these Tasks are estimates, and assume favorable conditions.  In particular, Task 3 will require

collaboration with involved building owners.  This task cannot begin though until the other tasks are completed.  This means that suitable buildings with agreeable managements must be located and available promptly at the conclusion of the other tasks.  Locating and arranging suitable buildings and maintaining this schedule for Task 3 may meet with difficulty.

| Task 1 - MVOC from culture | 7 | 10 mos total (these will overlap) |
|---|---|---|
|    - MVOC analysis/interpretation | 5 | |
|    - MVOC pattern analysis | 3 | |
| Task 2 - MVOC collection in chambers | 3 mos | |
| Task 3 - Field validation | 5 mos | |

## 4.4 GLOSSARY OF TERMS

**actinomycete** – bacteria that are multicellular (filamentous) rather than single celled

**bioaerosol** – aerosol composed of material that is biological in origin, including spores, pollen, organic dusts, insect debris, etc.

**biomass** – material that is biological in origin, organic material

**chemotype** – variants within a species that differ in chemical profile rather than morphology

**colonize** – start a new microbial colony; for example, when a spore lands on a suitable substrate, germinates, digests the substrate, grows and produces a colony of mold, and releases new spores

**cytotoxic** – property of being toxic to cells

**endotoxin** – an outer membrane component (lipopolysaccharide) of gram negative bacteria; the lipid portion is toxic, causing fever, and respiratory inflammation; endotoxin contamination is considered ubiquitous

**explant** – material removed from a culture plate

**genera** – plural of genus

**genus** – a level of taxonomic classification in biology above species,  hence closely related species are grouped into a genus

**GC/MS** – gas chromatograph / mass spectrometry

**hypha (plural: hyphae)** – thread-like filament composed of multiple fungal cells arranged end to end; the fungal "body" is a mass of vegetative hyphae, called a mycelium

**inflammatory mediator** - compound that induces inflammation

**in vitro** - literally "in glass" (cf in vivo) referring to reactions or events occurring in an artificial environment (such as a test tube or culture dish)

**LD$_{50}$** - lethal dose 50%, that is, the dose of a material that kills 50% of the test organisms or cells

**metabolite** - a chemical compound that is involved in a metabolic pathway

**microbial** - of, or pertaining to microscopic organisms, such as viruses, bacteria, fungi, or certain protists

**MVOC** - Microbial volatile organic compounds, i.e. volatile chemicals that are produced by micro-organisms

**mycelium** - (plural mycelia) the network of individual hyphae that comprise the body of molds and other fungi, also applied to actinomycetes

**propagule** – spore or other component such as hyphal fragment that can colonize a new substrate

**saprophyte** - an organism that lives on decaying organic material in contrast to a parasite, which lives on living tissue

**substrate** - as applied to microbial growth, substrate is the material that the microbes are living on, e.g. agar in a laboratory culture plate or damp building materials in a building

**spore** - a portion of the body of a mold that is produced and adapted for dispersal through time or space; generally spores reach new sites for colonization. Molds that occur in buildings typically produce spores that are dispersed in air through space, although some spores disperse through water and others are adapted to persist in the same place through long periods of time.

**taxa** - (sing taxon) any of the taxonomic levels of classification. Species are taxa, as are genera and families and orders. Sub-specific taxa include subspecies, varieties, and forma speciales

**viable** – alive

**VOC** - (volatile organic compound) a chemical that is volatile under ambient conditions